

SEQ ID No. 1:
 Human RANKL
 (aa 68)
FYFRAQMDPNRISEDGTCTYRILRLHENADFQDTTLESQDTKLIPDSCCRRIKQAFQGAVQKELOHQ
IVGSQHIREXZAMVGDGSMFLDAKREKLEAQPFAHLTINATDIPSGSHKVSLSSSWTHDRGWAKISNM
TFSNGKLIVNQDGFFYLYANICFRHHETSGDLATEYLQLMVTYTKTSIKLPSSSHLMKGGSTKXWS
GNSBFFHFTYSINVGGFFKLRSGEEETIEVSNPSLLDPDQDATYFGAKVRDID

Figure 1a

SEQ ID No. 2:

HIS Tag	TEV Protease	Human RANKL
<u>HISHHHHHHSSGGIIEVLFQGPKLEAQPAHLTINATDIPSGSHKVLSMYHDRGWAKISNM</u>	Cleavage Site (aa 159)	
SNGKLIVNODGFFYLYANICFRHHETSGDLATEYLQLMVTYTKTSIKLPSSSHLMKGGSTKX		
WSGNSEFHFTYSINVGGFPKLRSGEEETIEVSNPSLLDPDQDATYFGAKVRDID		

Human RANKL
 (aa 317)

Figure 1b

SEQ ID No. 3:

Mouse RANKL
(aa 68)
FLYFRAQMDPNRISEDGTCTYRILRLHENADLQDSTLESEDTLIPDSCCRRIKQAFQGAVQKE
LOHIVGPORFSGPAMMESSWLDVAGRGKPEAQFAHLTINAAISPSSGHKVTLSSWYHDRG
WAKISNMFLSNGKRLRVNQDGFFYLYANICFRHHETSGVPTDYLQLMVTYVVRTSIKIPSSHN
LMKGGSTKNSNSEFHFTYSINVGGFFKLRAGEEISIQVSNPSLLDPDQDATYFGAKVQDI
D (Mouse RANKL aa 316)

Figure 1c

Human: 159 KLEAQPFPAHLTINATDIPSGSHKVSLSSSWYHDRGWAKISNMTFSNGKLIWNQDGFYLYA 218
K EAQPFPAHLTINA IPSGSHKV+LSSWYHDRGWAKISNMT SNGKL VNQDGFYLYA
Mouse: 158 KPEAQPFPAHLTINAASIPSGSHKVTLSSWYHDRGWAKISNMTLSNGKLRVNQDGFYLYA 217

Human: 219 NICFRHHETSGDLATEYLQLMVVVTKTTSIKIPSSHTLMKGGSTKYWSGNSEFHFYSINVG 278
NICFRHHETSG + T+YLQLMVVV KTSIKIPSSH LMKGGSTK WSGNSEFHFYSINVG
Mouse: 218 NICFRHHETSGSVPTDYLQLMVVVKTTSIKIPSSHNLMGGSTKWNWSGNSEFHFYSINVG 277

Human: 279 GFFKLRSGEEISIEVSNPSSLLDPDQDATYFGAFKVRDID 317
GFFKLR+GEEISI+VSNPSSLLDPDQDATYFGAFKV+DID
Mouse: 278 GFFKLRAGEEEISIQVSNPSSLLDPDQDATYFGAFKVQDID 316

Figure 2a

T173R/C221S
T173E/C221S
S183R/C221S
I207D/C221S
I207E/C221S
I207K/C221S
I207R/C221S
A232K/C221S
I247A/C221S
I247D/C221S
I247E/C221S
I247K/C221S
I247Q/C221S
I247R/C221S
C221S/T173R/S183R/I207R/I247R

Figure 2b

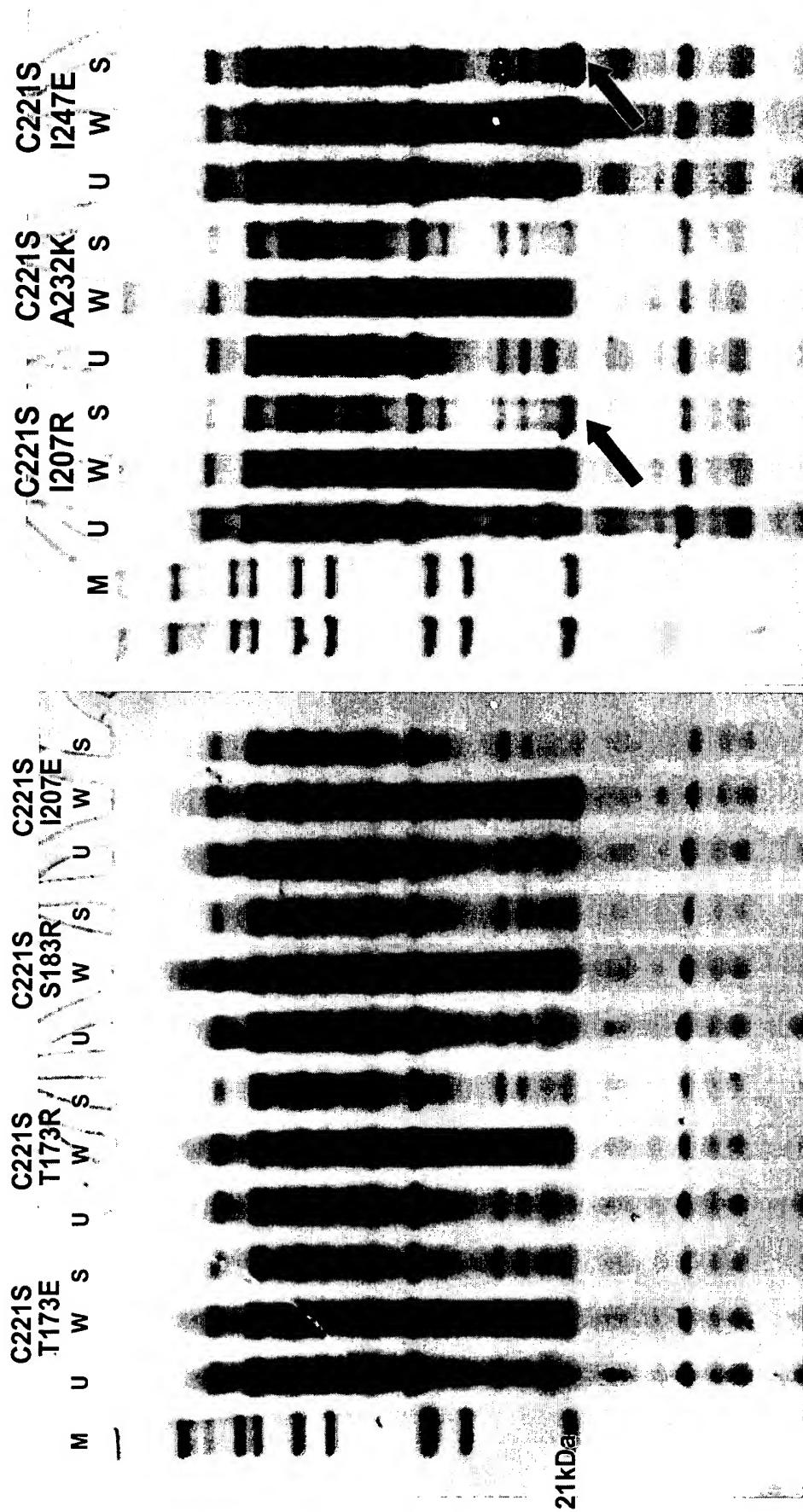


Figure 3

Coomassie Stained Gels

Western blot probed with anti-HIS

Coomassie Stained Gel

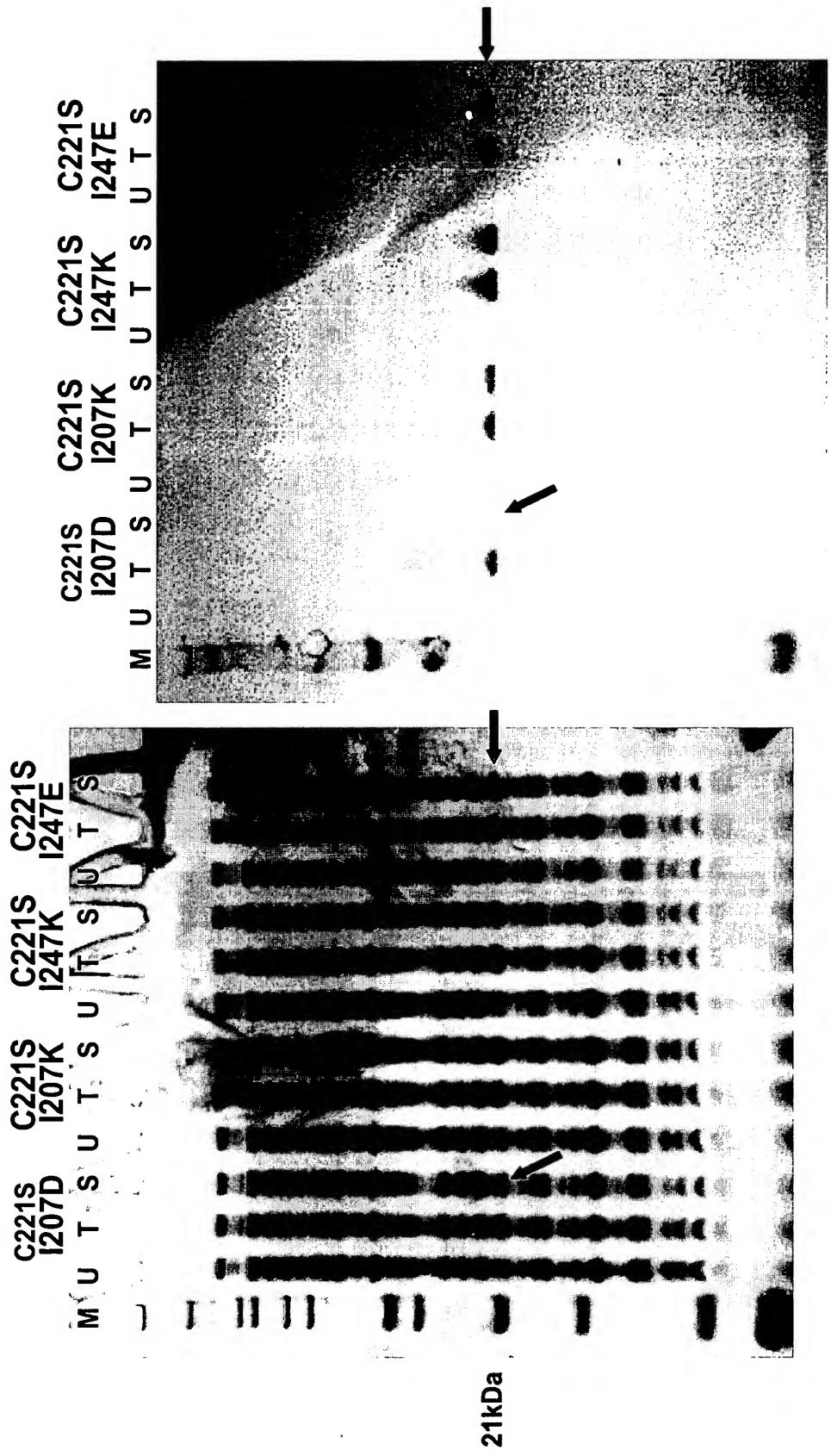
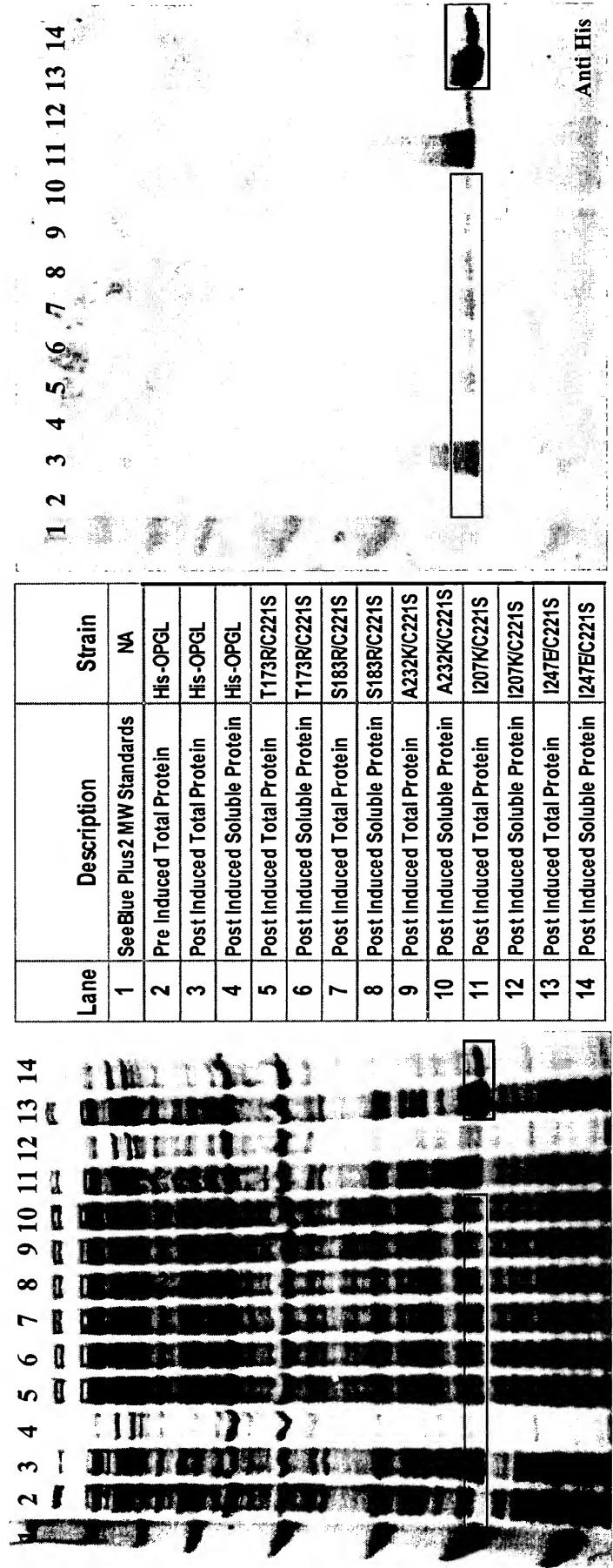


Figure 4



Coomassie Stained Gel

Western blot probed with anti-His

Figure 5

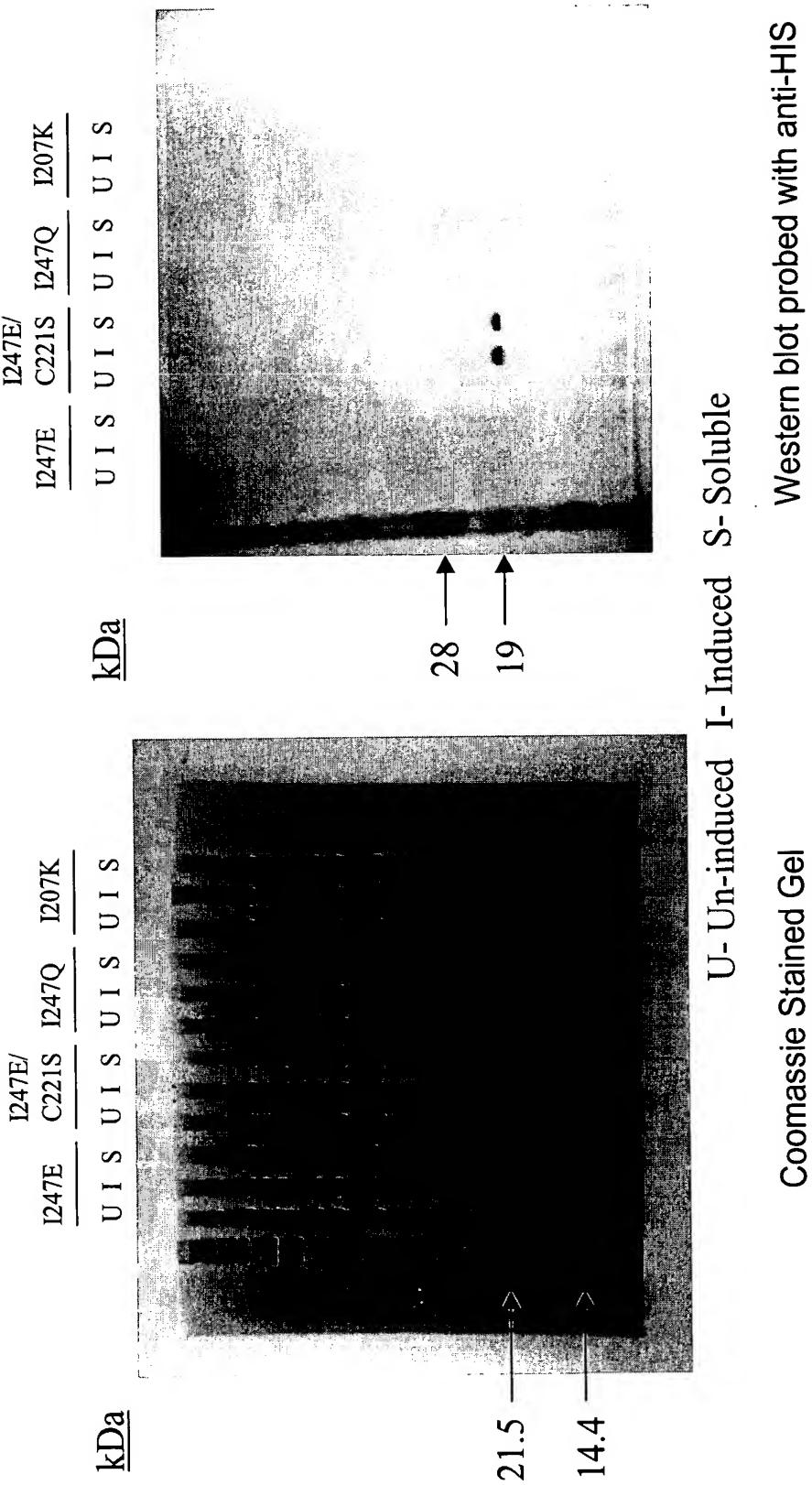
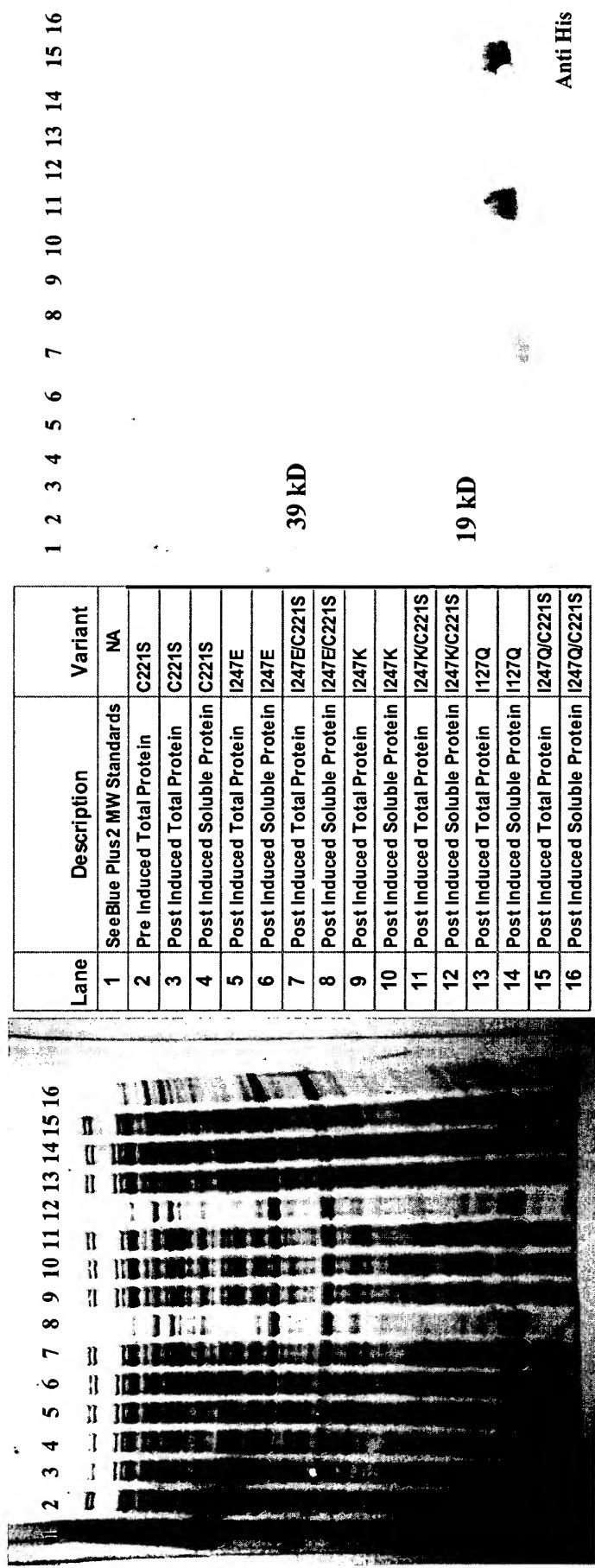


Figure 6

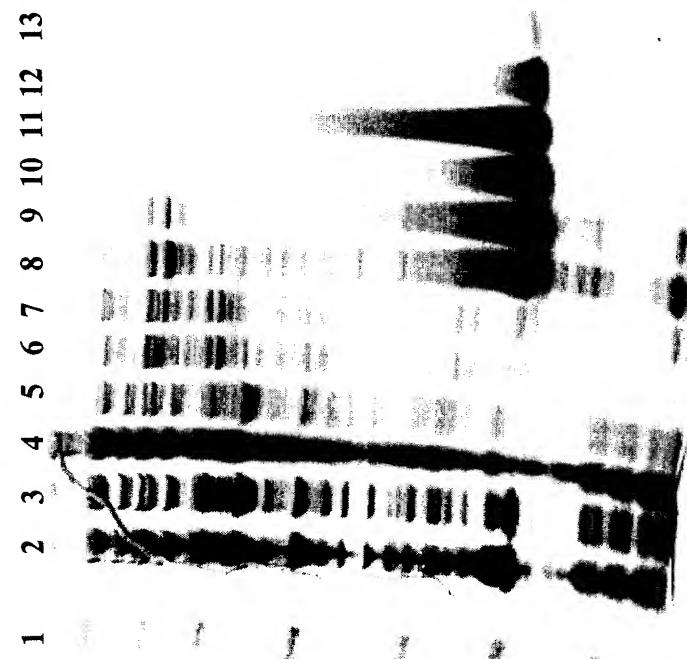


25C, 0.5 mM IPTG, 4 hr induction
Coomassie Stained Gel

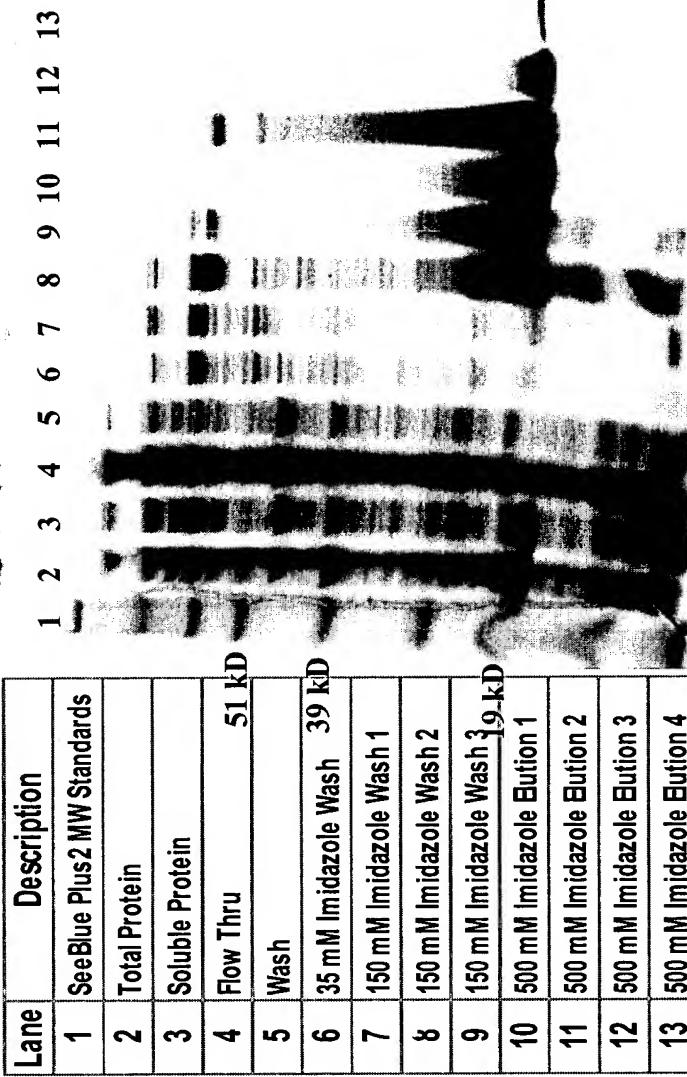
Western blot probed with anti-HIS

Figure 7

I247E/C221S



I247D/C221S



25C, 0.5 mM IPTG, 4 hr induction
Coomassie Stained Gel

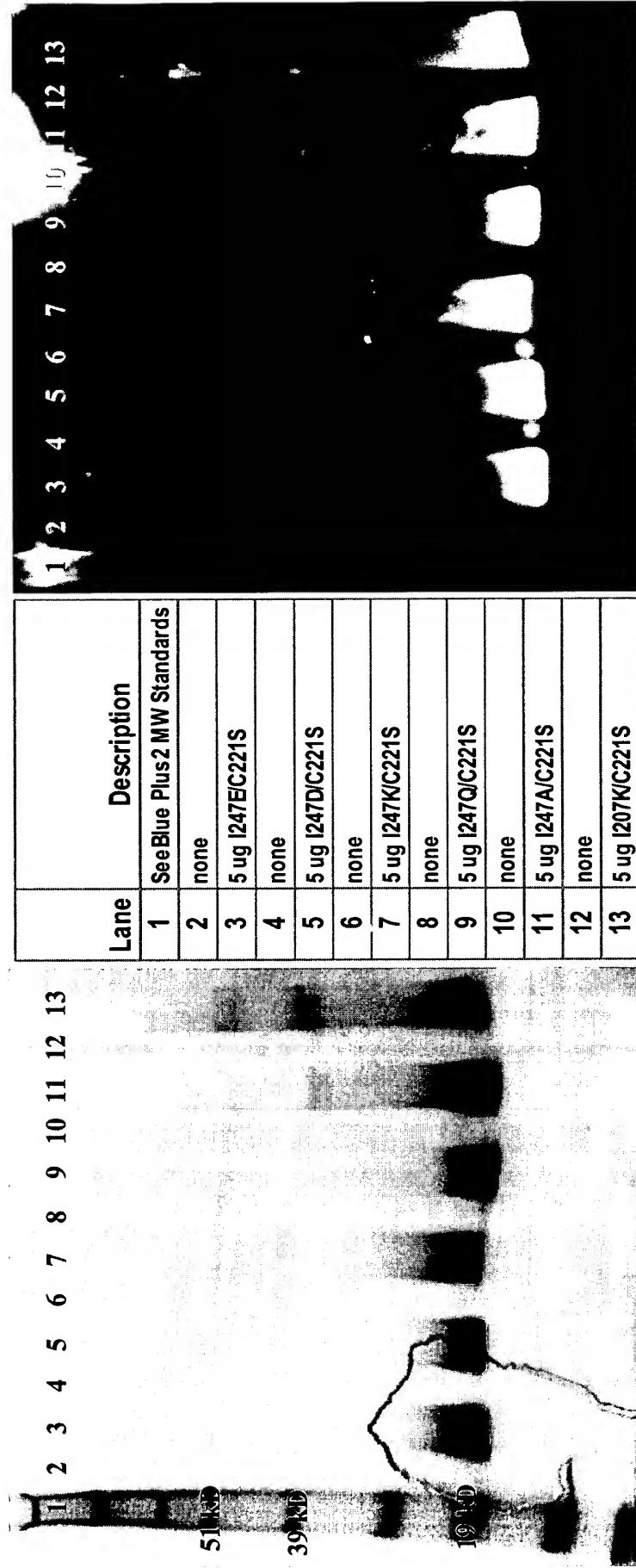
Figure 8

Coomassie Stained Gel

Figure 9

Western blot probed with anti-HIS

Coomassie Stained Gel



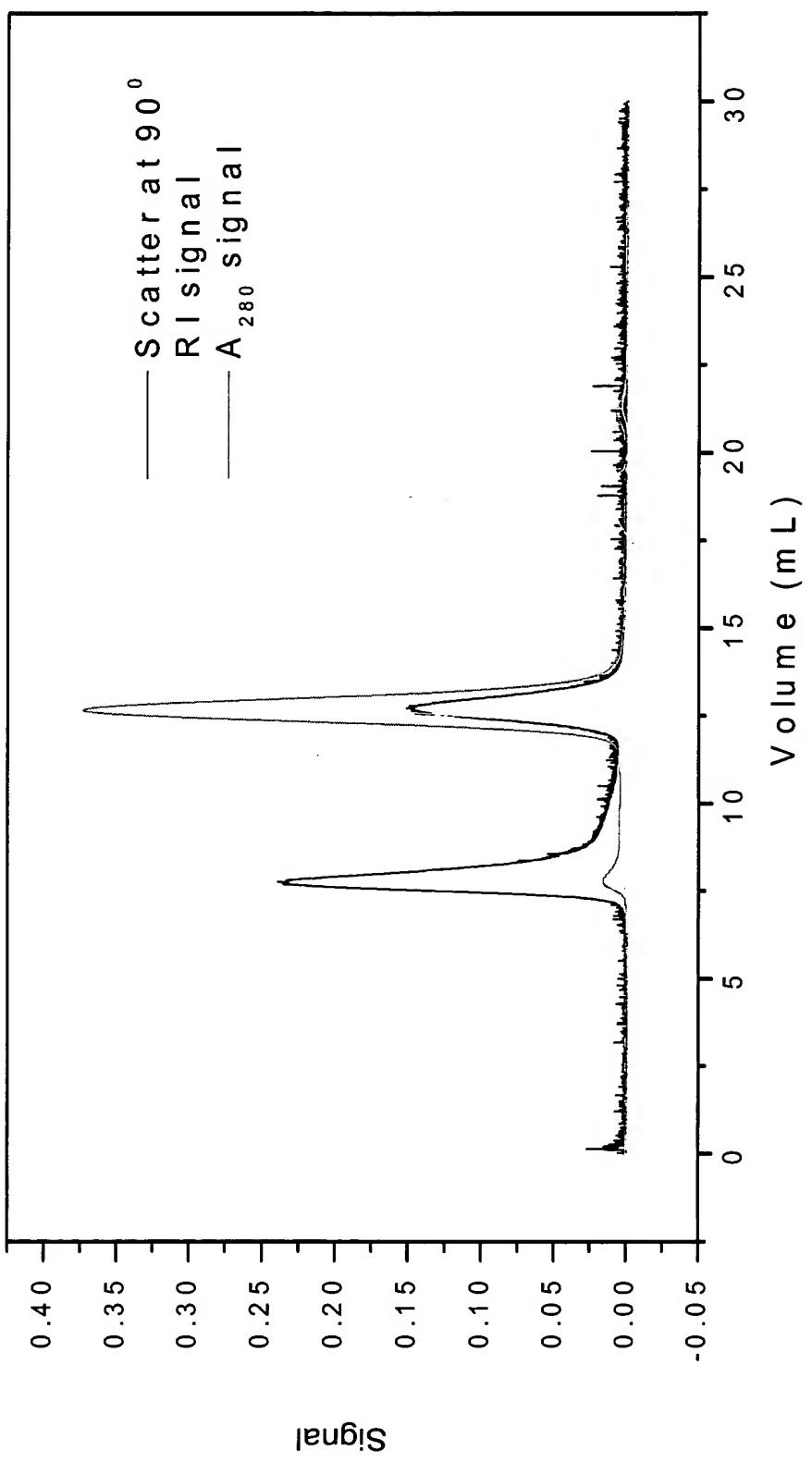


Figure 10

Figure 11

Variant	Mw (Kd)	% Trimer	% Oligomer	Expression
C221S/I247E	65	97	3	1.4 mg/g
C221S/I207K	68	90	10	1.2 mg/g
C221S/I247K	71	84	16	1.2 mg/g
C221S/I247Q	69	91	9	1.1 mg/g
C221S/I247A	67	92	8	1.4 mg/g
C221S/I247D	68	92	8	1.3 mg/g

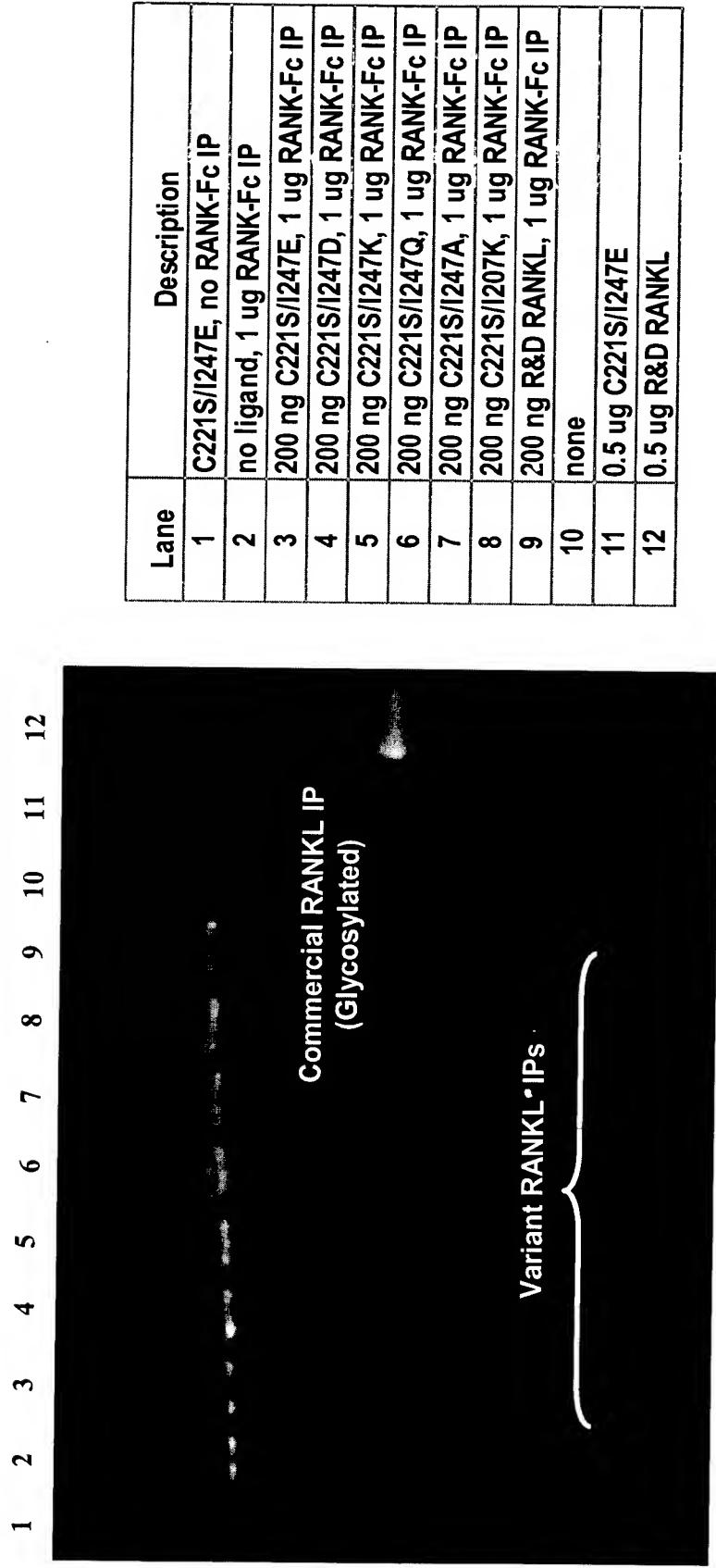


Figure 12

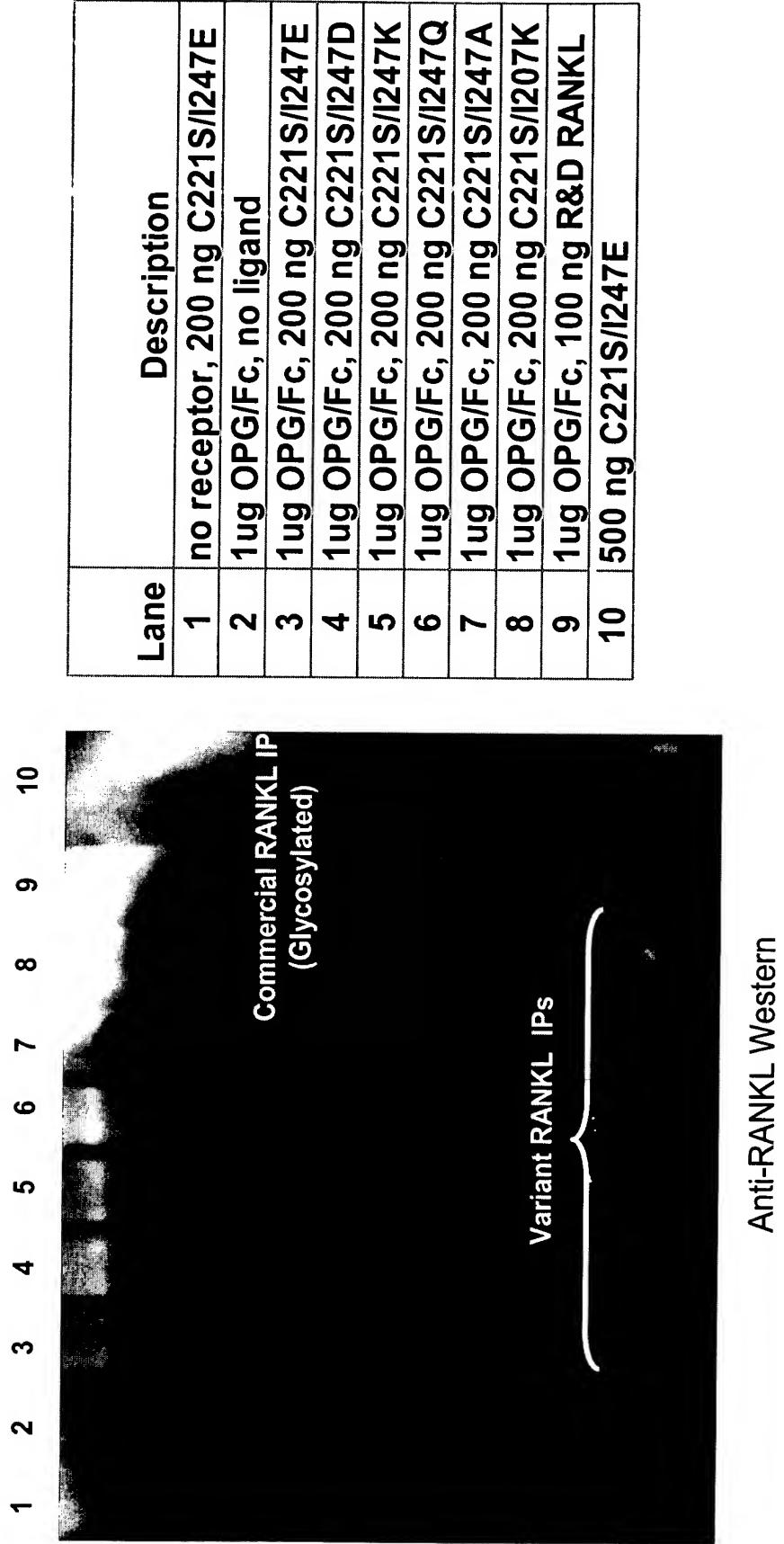


Figure 13

Figure 14

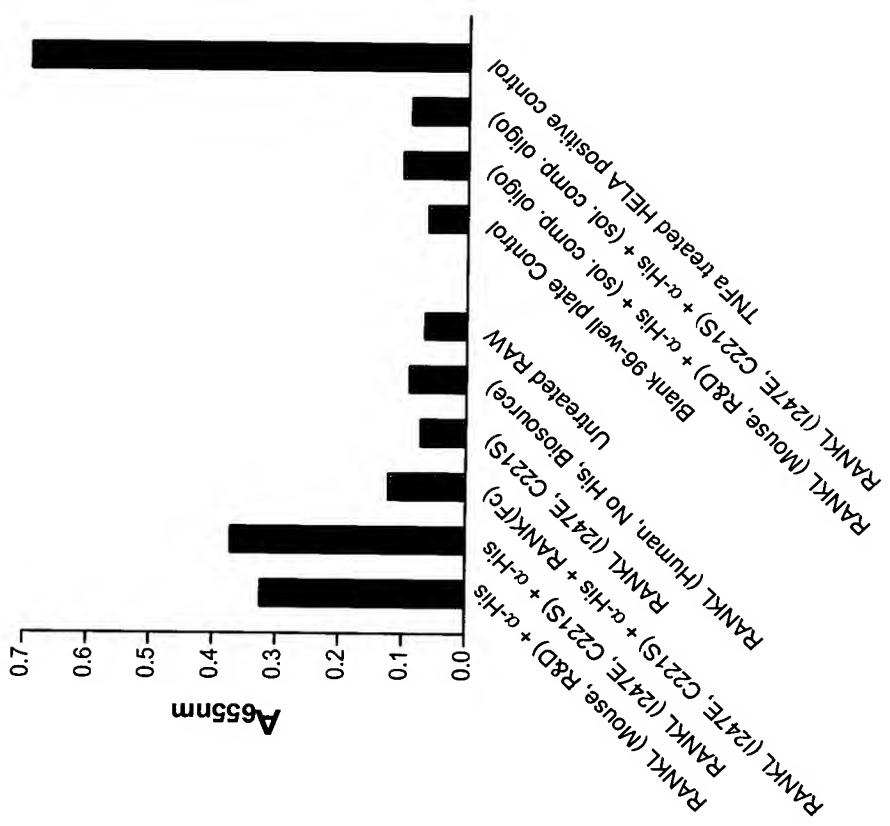
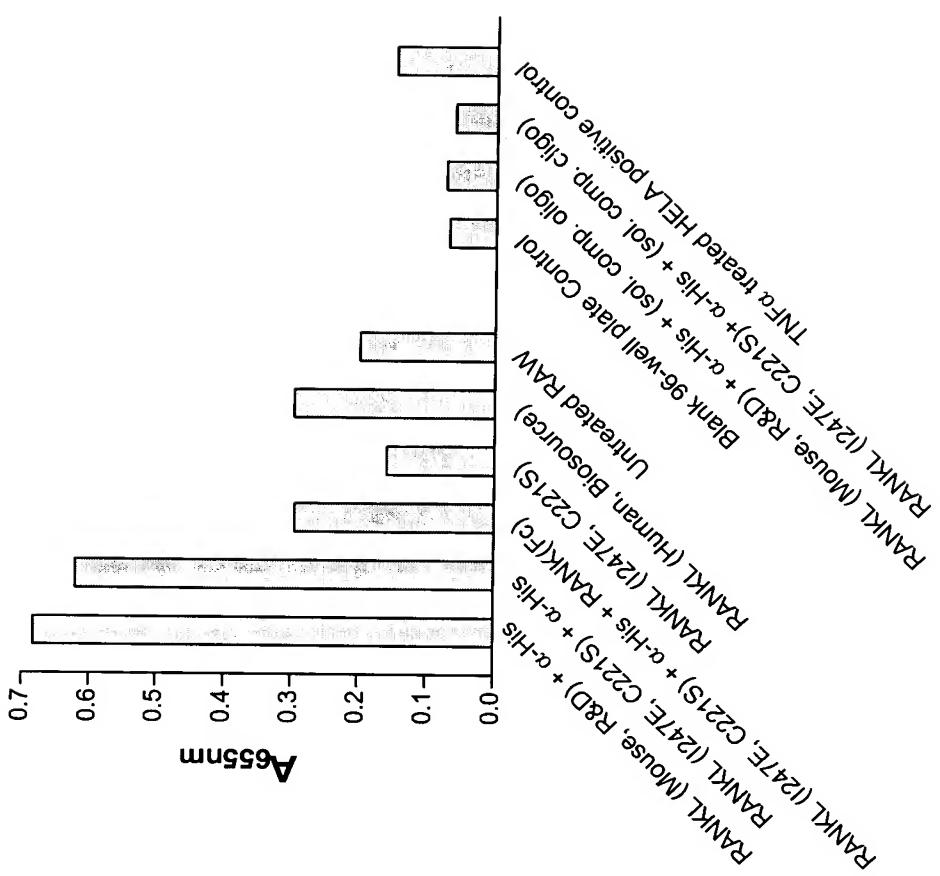
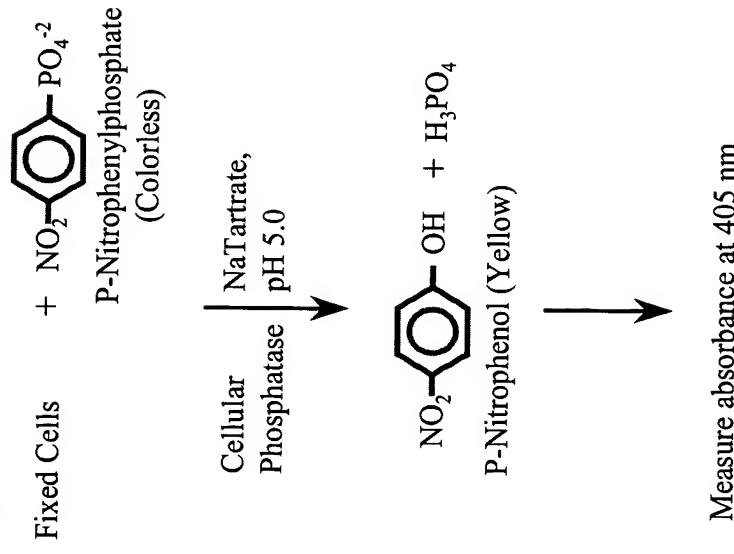


Figure 15



TRAP Solution Assay



TRAP Staining Assay

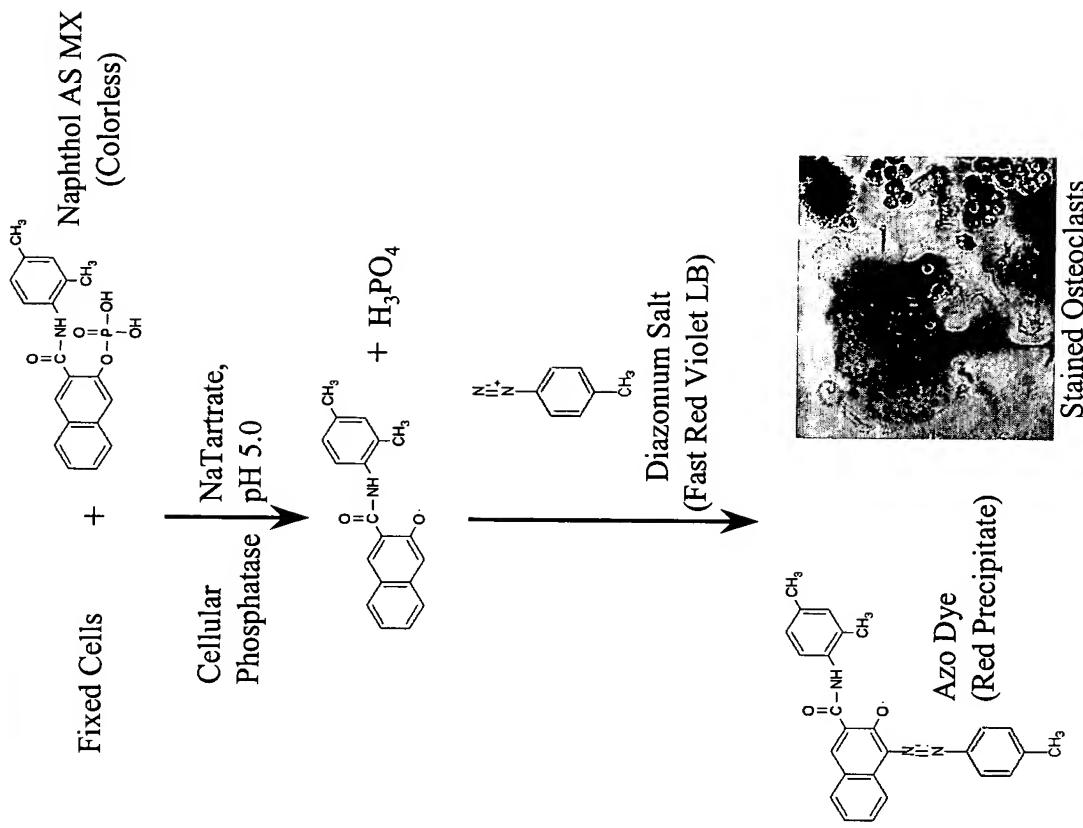


Figure 16

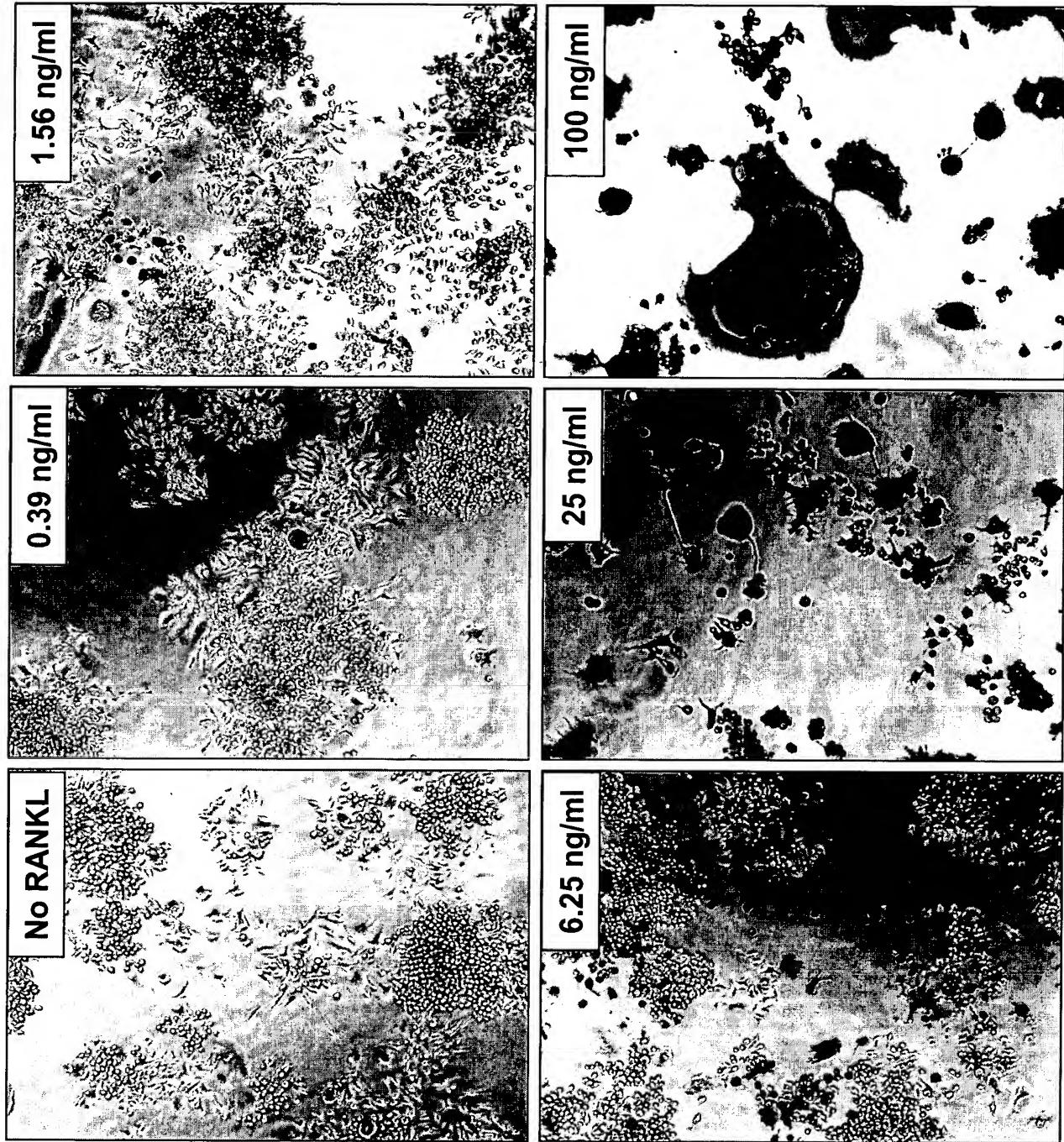


Figure 17

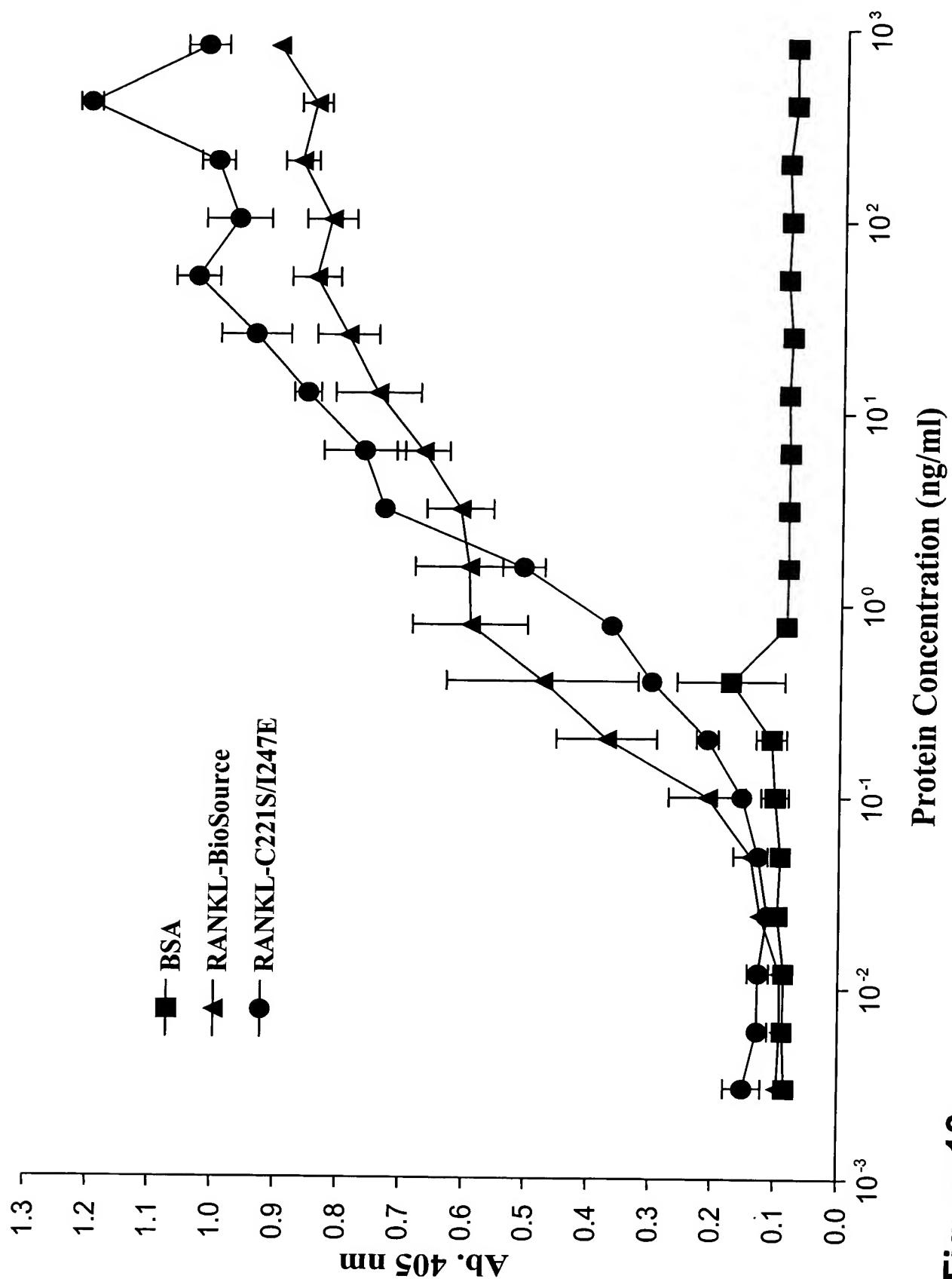


Figure 18

Protein Concentration (ng/ml)

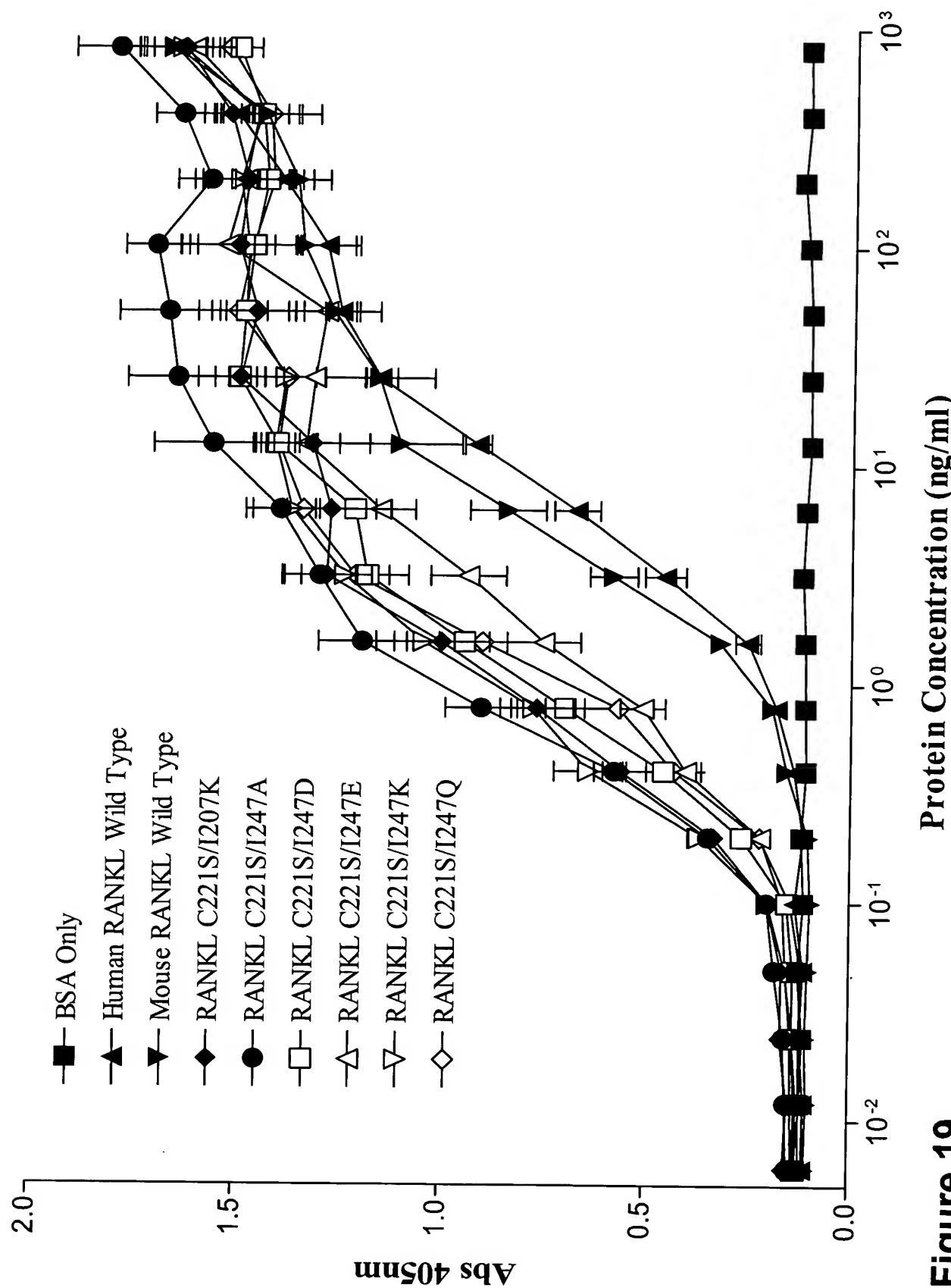


Figure 19

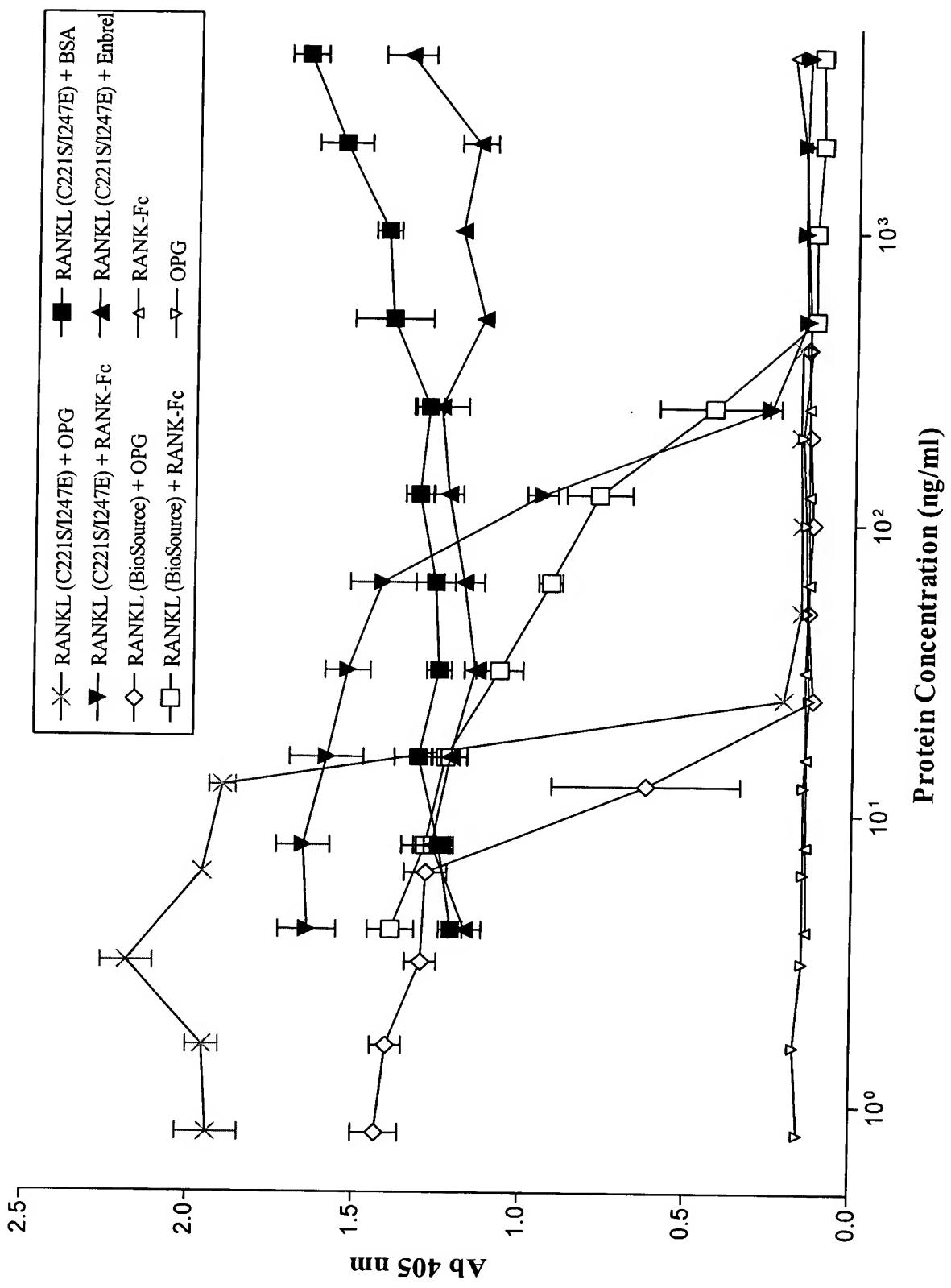


Figure 20

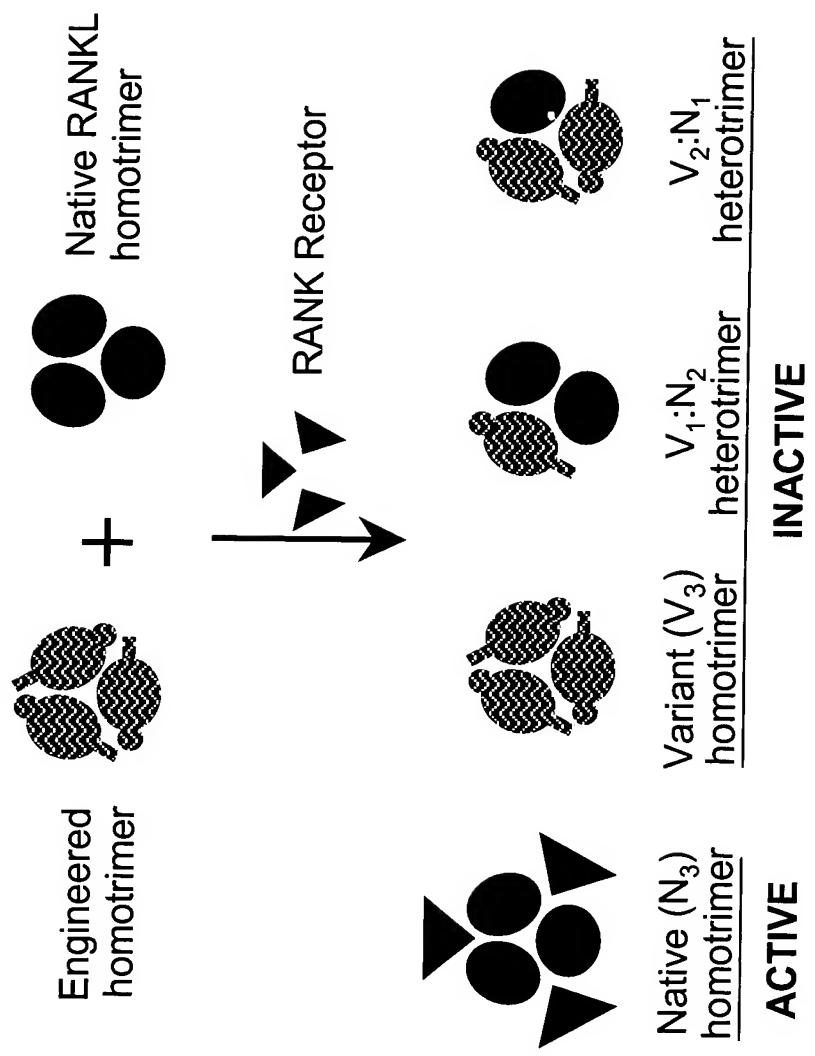


Figure 21

Single Chain Dominant Negative Polypeptides

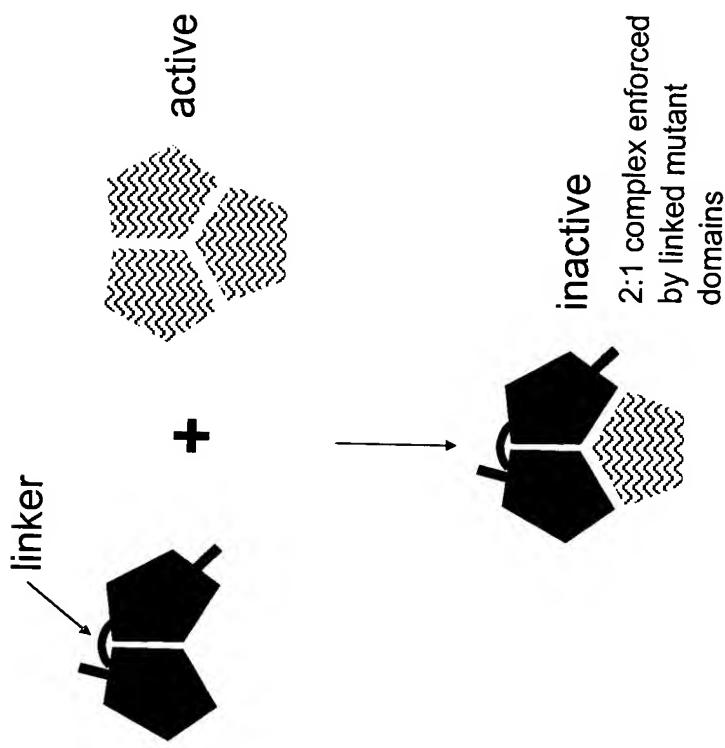


Figure 22

G191A	I249R	D1190T-K248E
A1172R	R223Q	H253T
A1172Q	R223E	H253S
S1179E	R223M	S268D
S1179D	H225R	E269R
H1180E	H225N	E269Q
H1180N	H225T	E269T
H1180S	H225E	E269K
K1181Q	E226Q	F270V
K1181E	E226R	F270E
K1181R	E226D	F270T
S1183K	T227K	F270K
S1183E	T227Q	R284E
Y1188F	S228H	S297Q
Y1188M	S228Q	S297E
Y1188Q	S228E	S297D
Y1188E	S228D	D300N
H1189E	T233R	D302E
H1189A	T233D	D302Q
H1189R	T233E	D302H
D1190Q	Q237K	R191K-I249R
D1190T	Q237E	Q237T
R1191K	Q237T	K248E
R1191E	K248E	I249Q
R1191Q	I249E	H225E-E226D-F270T

Figure 23

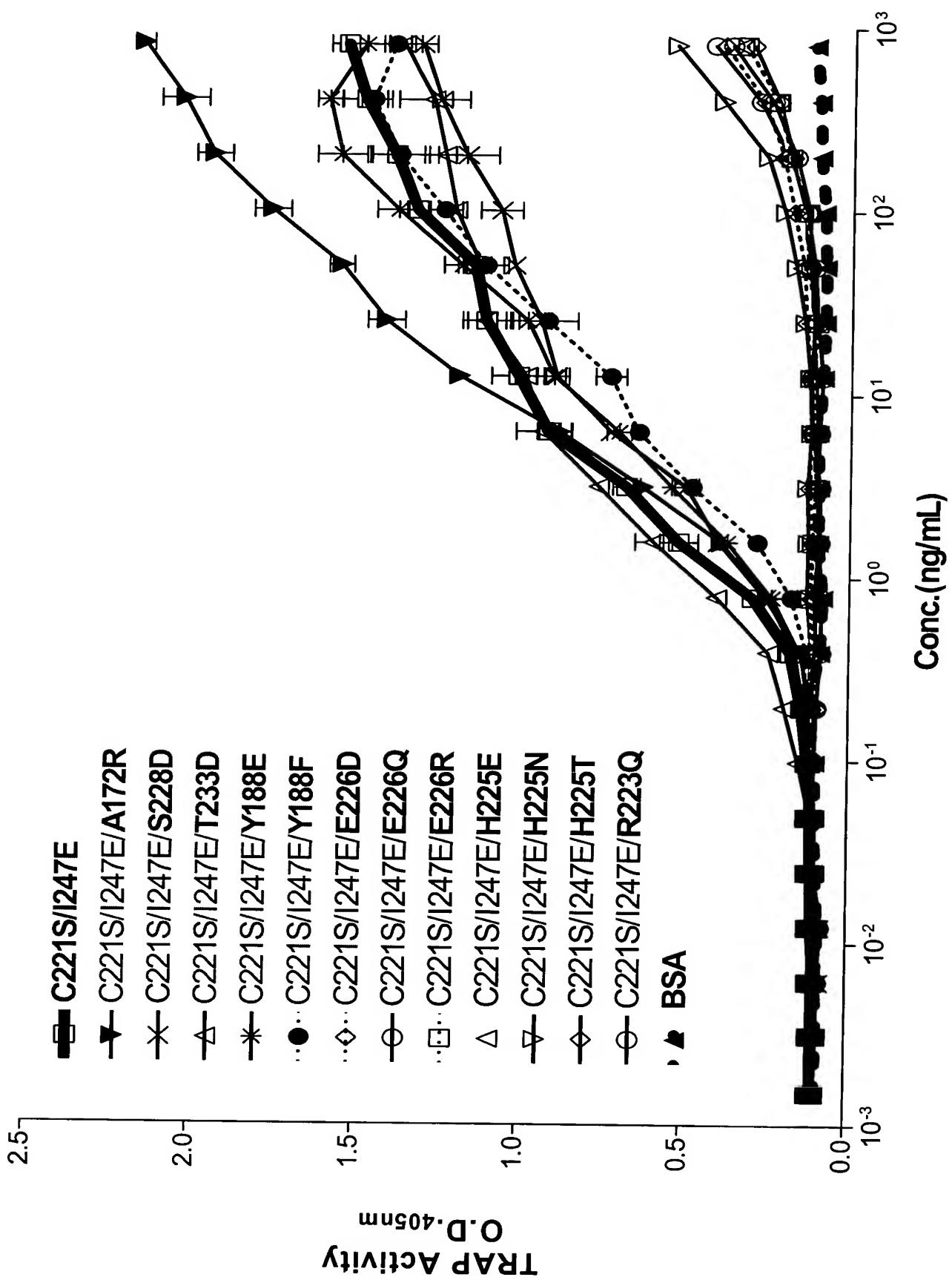


Figure 24

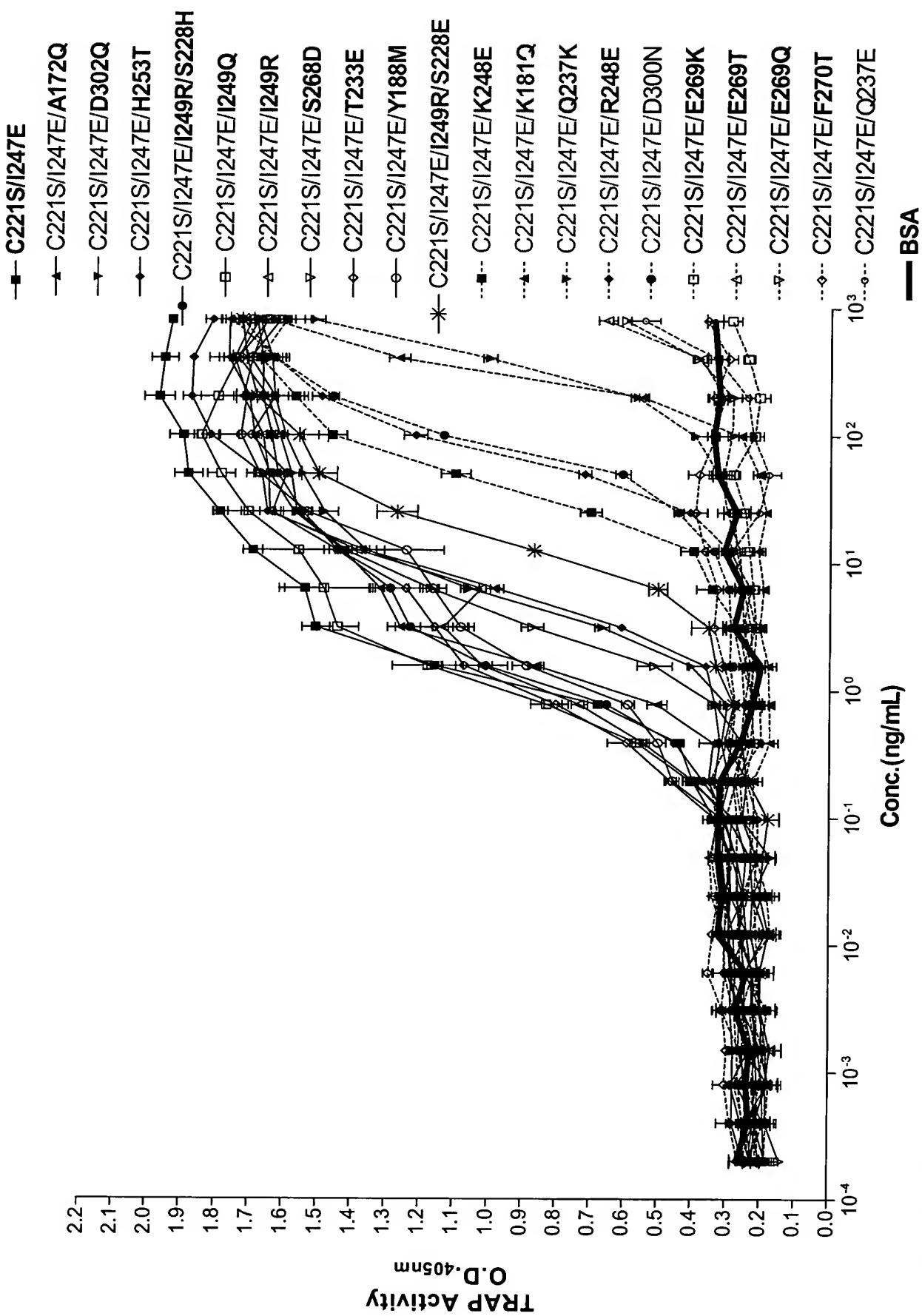


Figure 25

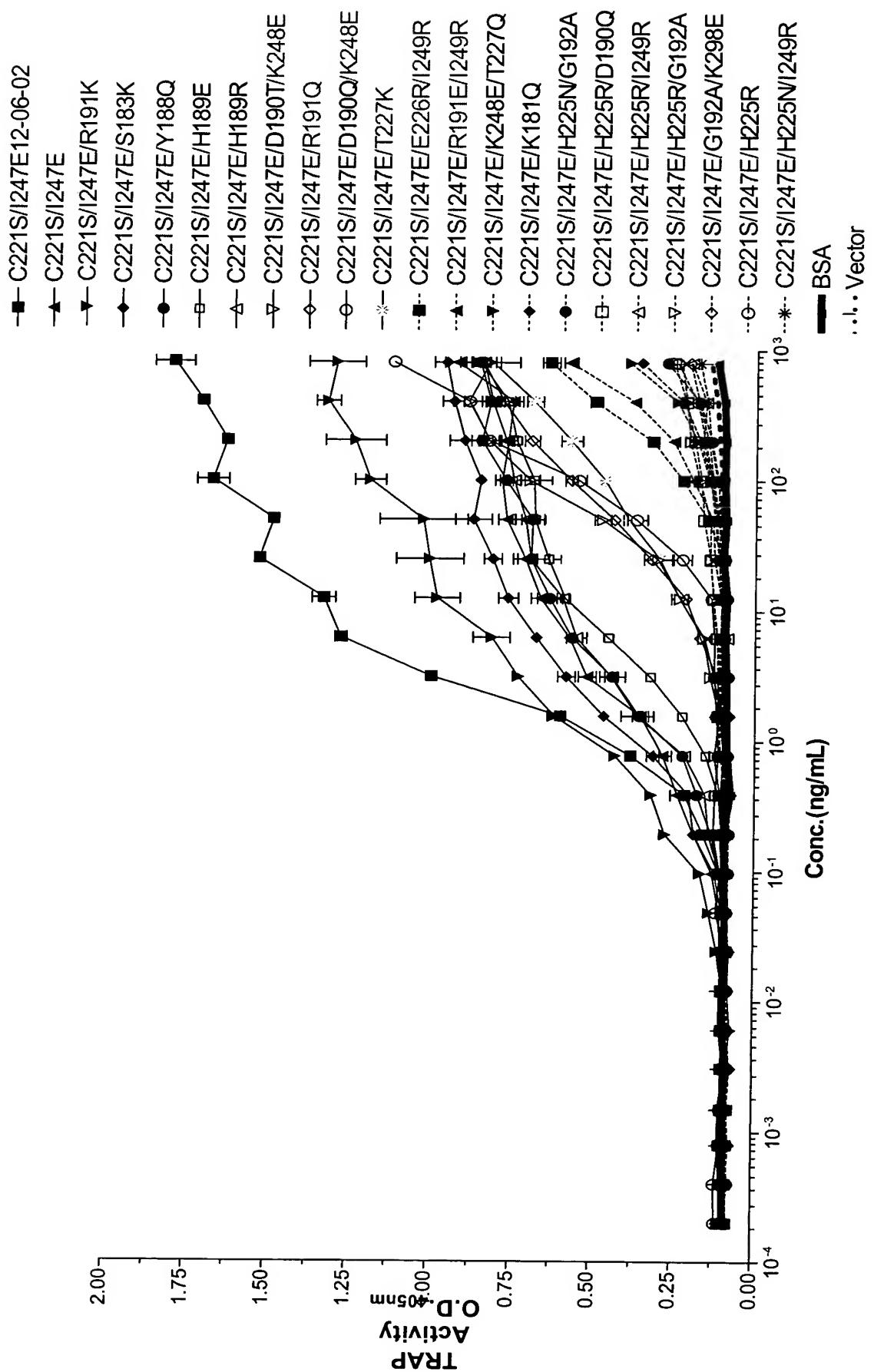


Figure 26

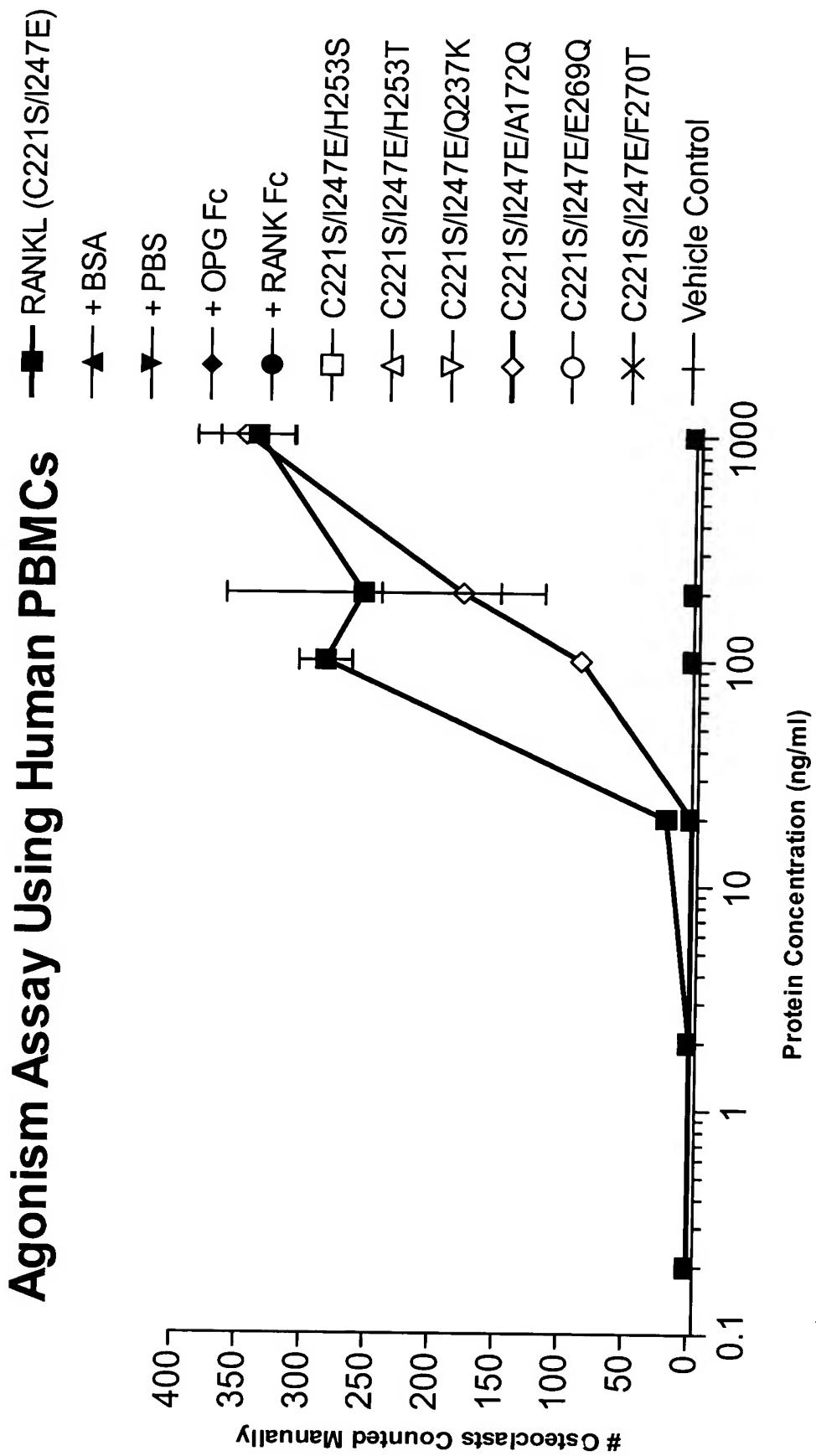


Figure 27

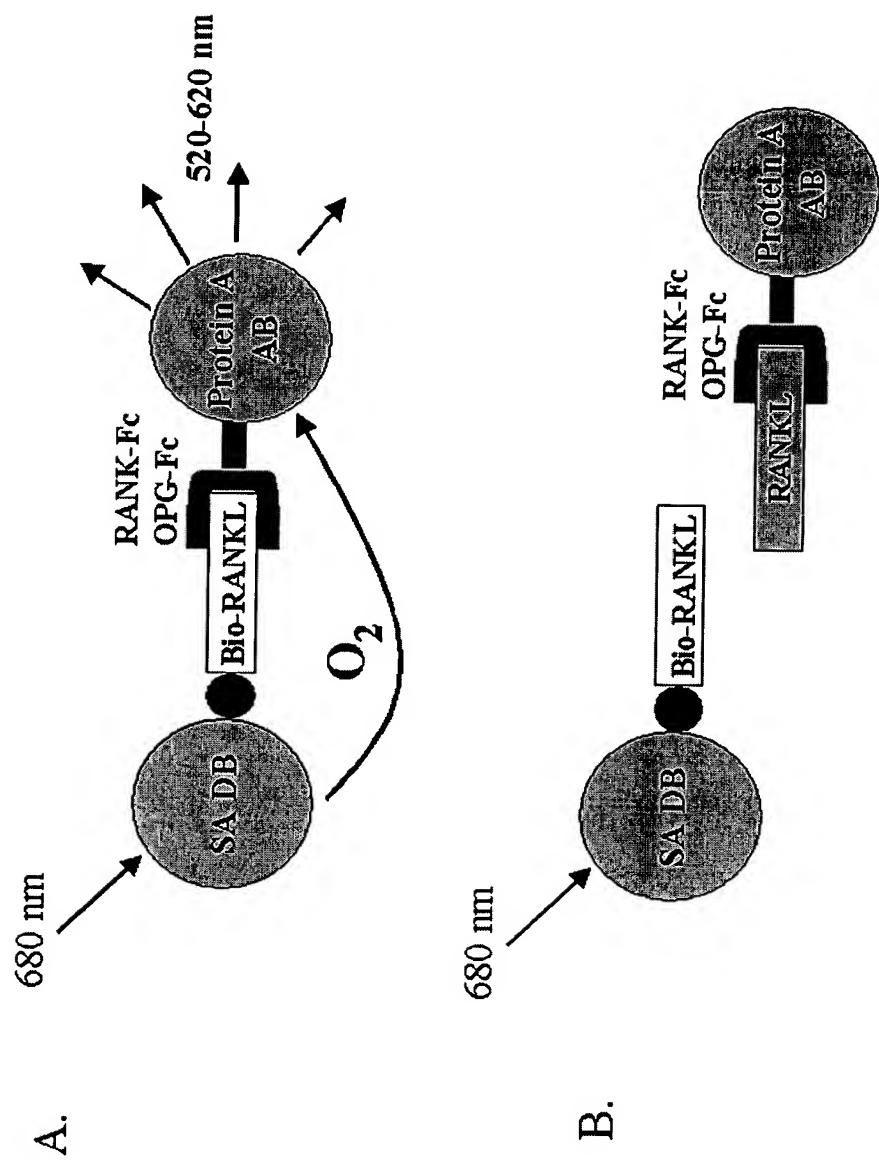


Figure 28

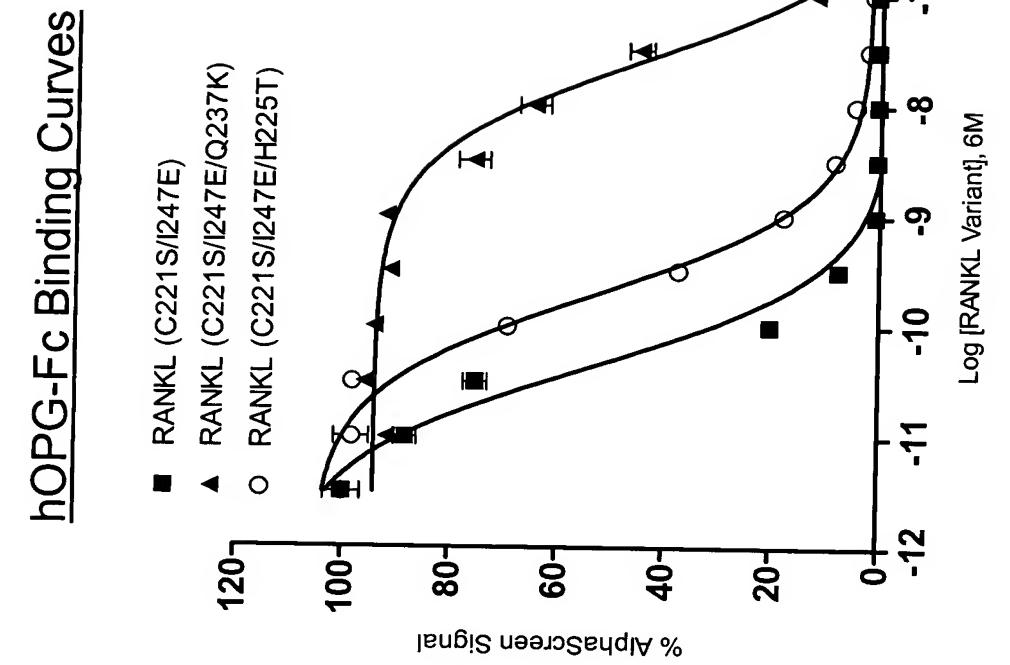
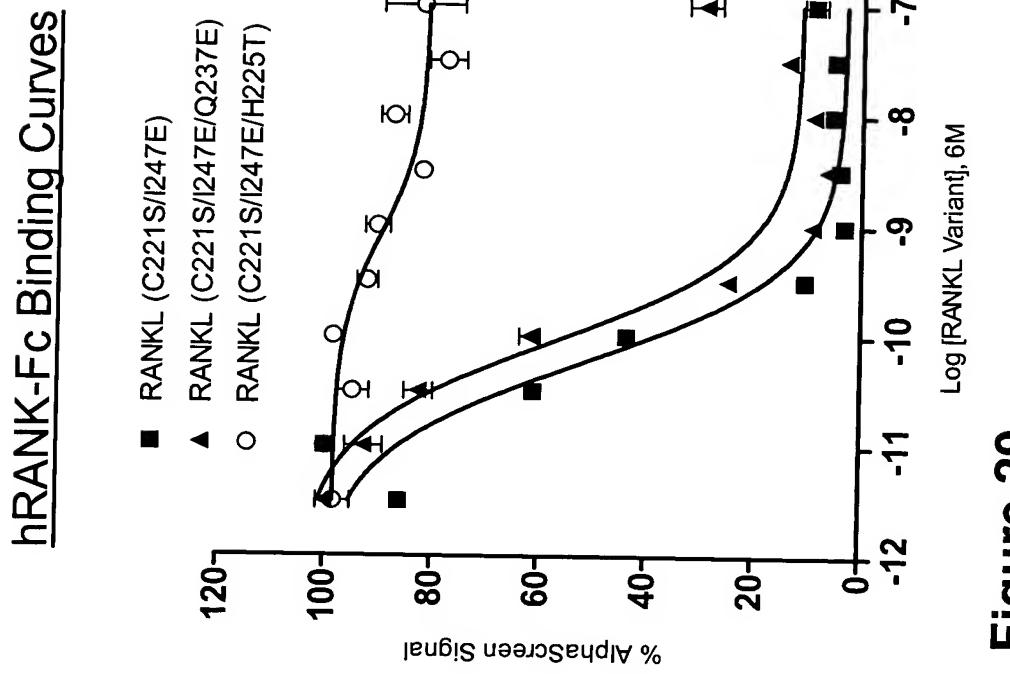


Figure 29

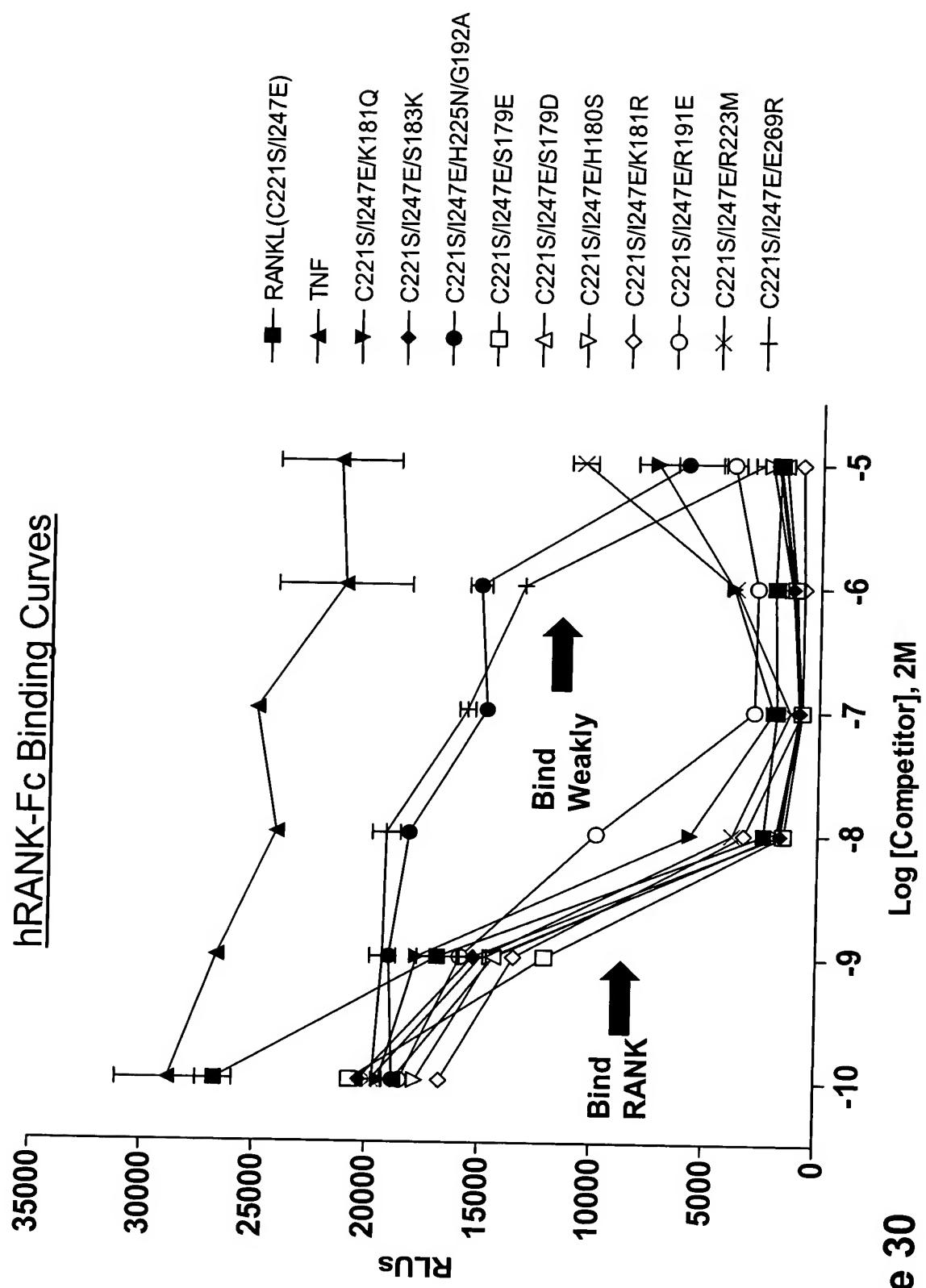


Figure 30

hOPG-Fc Binding Curves

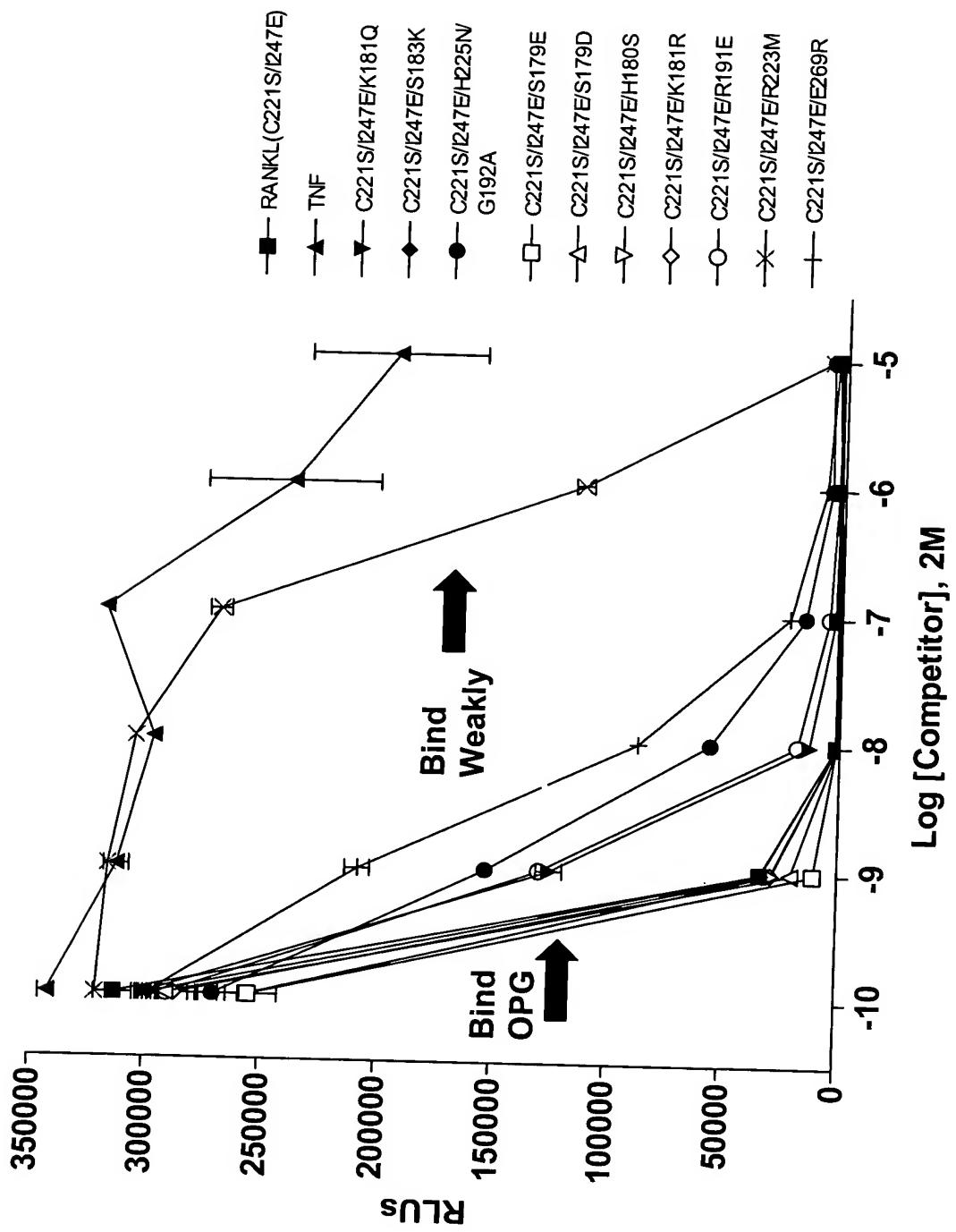


Figure 31